Table 1. Biossay results of compounds **1–3** against *Tenebrio molitor* pupae

Run	Compound	Concentration (mg litre^{-1})	N	ΔN	$\Delta N/N$,%
1	**2**	1000	3.1	0.36	11.7
2	**3**	1000	3.5	0.80	22.8
3	**1**	1000	6.0	0.55	9.1
4	Altozid-SR-10	1000	9.0		

individual, the weighted average score (N) for compounds **1–3** was determined using the formula:

$$N = \frac{x_1 \cdot 1 + x_2 \cdot 2 + x_3 \cdot 3 + \cdots + x_n \cdot n}{A}$$

The average error (ΔN) was calculated by the formula:

$$\Delta N = \frac{x_1|N-1| + x_2|N-2| + \cdots + x_n|N-n|}{A}$$

1, 2, 3, … n – score from zero to nine,

x_1, x_2, x_3, … x_n – number of individuals with each score,

A – total number of individuals.

The results of bioassays are presented in Table 1.

In contrast to 1,2-, 1,5-, and 1,6-disubstituted benzimidazoles reported by Kuwano,[1] we studied 1-terpenylbenzimidazole without substituents in the 2, 5 or 6 positions of the benzimidazole ring. Furthermore, the structure of the terpenyl moiety differed from that of the natural terpenes by the position of the methyl groups and of the double bond. Benzimidazoles **1–3** showed lower insecticidal activity than that of the methoprene standard. The isomer of geranylbenzimidazole, compound **2**, showed poor activity in this series; the activity of the linalylbenzimidazole isomer, compound **3**, was greater. Imagos, resulting from the action of compounds **2** and **3** on pupae, had pupal cuticle on some parts of their bodies. With the introduction of methoxy group in the 7-position of the terpenyl chain the insecticidal activity was expressed; thus the treatment of pupae with compound **1** gave living pupal-imaginal intermediates in which abdomens had pupal cuticle and wing buds were greatly reduced.

4 CONCLUSIONS

It was found that the monoterpenylbenzimidazoles **1–3** with 2,7-dimethyloctane skeleton of the terpenyl moiety are insect growth regulators with juvenile hormone activity.

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Photolysis of imidacloprid (NTN 33893) on the leaf surface of tomato plants

Konrad Scholz* and Fritz Reinhard

Bayer AG, Crop Protection Development Institute for Metabolism Research and Residue Analysis, D-51368 Leverkusen, Germany

Abstract: The photolytic behaviour of the insecticide imidacloprid on the surface of tomato leaves as a result of exposure to natural sunlight was investigated. Photodegradation in sunlight was rapid and the degradation products (⩾10%) were similar to those found in plant degradation studies.

Keywords: imidacloprid; photodegradation; tomato

An imidacloprid spray solution (0.1 mg ml^{-1}), containing [*methylene*-^{14}C] imidacloprid (Fig 1; sp act 4.64 MBq mg^{-1}), was prepared by adding labelled compound (106 µg; 492.26 kBq), thoroughly mixed with the formulants, to a commercial 200 g litre^{-1} SL formulation, (Confidor® SL 200), diluting with water and thoroughly agitating the mixture in an ultrasonic bath. The identity of the imidacloprid was verified both before and after application.

In practice, tomato leaves are sprayed to run-off with the formulation at 0.1 mg AI ml^{-1}. In the present work, one leaf (area *c* 30 cm^2) of each plant received 10 droplets of the spray solution (total 2.5 µl) which were then distributed over the leaf surface as a thin film, using a rubber-tipped wiper (Fig 2). Measurement of the radioactive content of the applied spray solution and the wiper indicated that each leaf received slightly less than 2.5 µg imidacloprid.

Experiments 1 and 2 were performed outdoors in Monheim, Germany (45 m above NN; 51°4′ latitude North, 6°55′ longitude East) 3 m in front of a greenhouse. The plants were arranged so that the treated leaves faced south. The incident radiation was measured at a point corresponding to the position occupied by the treated leaves, at 300–800 nm (Radialux) in Experiment 1 and 300–400 nm (UV-sensor) in Experiment 2. Experiment 3 (the dark control) was performed in the greenhouse, the treated

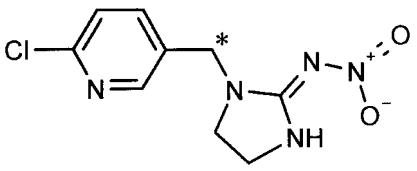

Figure 1. ^{14}C-labelled imidacloprid used in this work; * = labelling position.

* Correspondence to: Konrad Scholz, Bayer AG, Crop Protection Development Institute for Metabolism Research and Residue Analysis, D-51368 Leverkusen, Germany
E-mail: KONRAD.SCHOLZ.KS@bayer-ag.de

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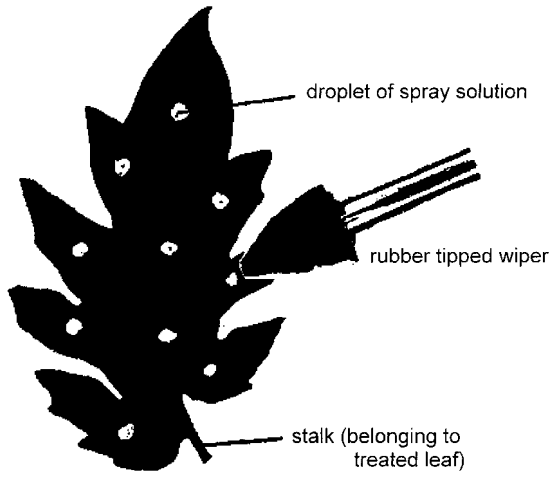

Figure 2. Application of imidacloprid to tomato leaves.

leaves only being contained inside a box made from black paper.

At each sampling date, the treated leaf, together with one untreated leaf [to evaluate the control (background) radioactivity] were removed and shaken with water (2×50 ml) and then with methanol (2×50 ml). The washed leaves were combusted to determine the non-extracted radioactivity. The water and methanol extracts were subsequently combined and the combined extract was applied as a band to a TLC plate, together with appropriate standard compounds for reference. TLC was performed at *c* 20 °C using (a) Silica gel 60 plates with ethyl acetate + toluene + methanol + acetic acid (80 + 20 + 20 + 1, by volume) as eluant and (b) RP-18 plates with acetonitrile + methanol + water + acetic acid (40 + 30 + 10 + 1, by volume) as eluant. The treated leaves each received *c* 2.4 μg imidacloprid per leaf, corresponding to 11 kBq radioactivity. The individual balance of recovered radioactivity varied from 94.7 to 106.3% between individual leaves over the incubation period (applied radioactivity = 100%). The non-extracted radioactivity amounted to $\leqslant 4.4\%$, indicating that translocation of imidacloprid from the treated leaves was slow. No device to trap volatile compounds was used but the complete mass balance indicated that the amount of volatiles must have been zero or very small.

The first water extract of a treated leaf contained *c* 80% of the applied radioactivity. This wash was colourless, suggesting that it contained the material that remained on the leaf surface during the experiments. By contrast, the methanol washes were green-coloured, indicating that they contained the material which had penetrated into the plant tissue. Data obtained from the two chromatographic systems were comparable. Besides unchanged imidacloprid, they showed the presence of up to 14 different metabolites, four of which amounted to $\geqslant 10\%$ during the study: these were the metabolites BNF 5540B, WAK 3839, BNF5529A and DIJ 9817 (Fig 3) which are known to be metabolites of imidacloprid in plant systems. The other, minor, metabolites ($\leqslant 3.8\%$ of the applied radioactivity) were not identified. The scheme shown in Fig 3 proposes that the breakdown of imidacloprid

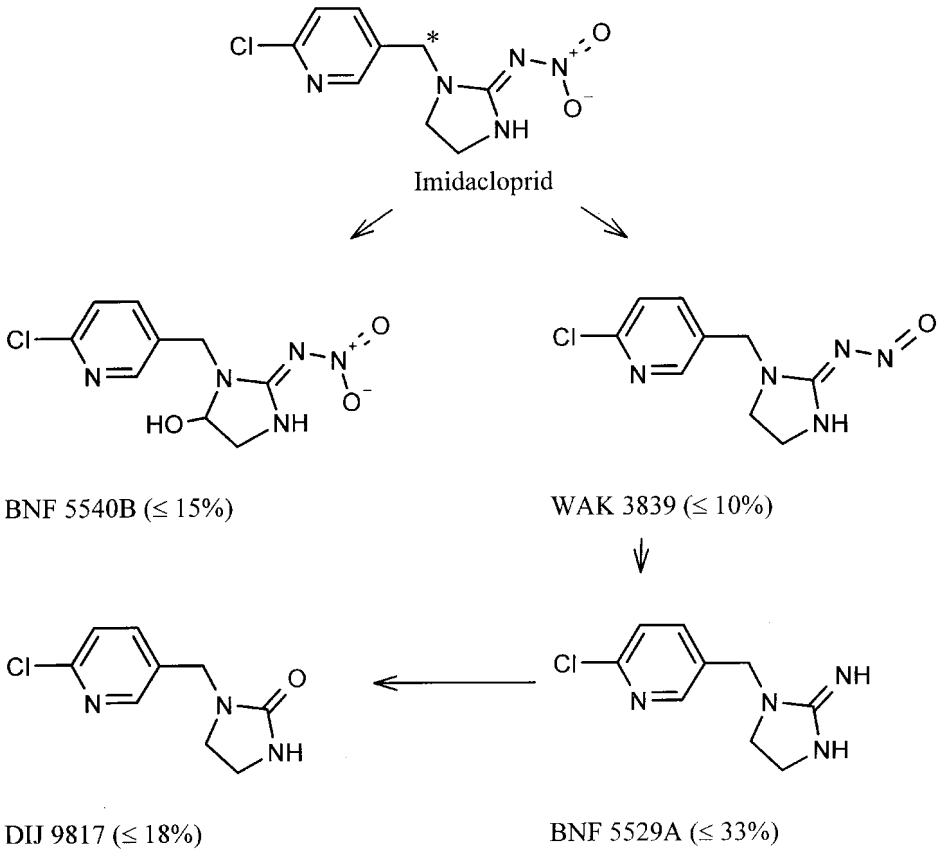

Figure 3. Photolysis of [*methylene*-^{14}C]imidacloprid on tomato leaves (proposed scheme for the degradation pathway); * = labelling position.

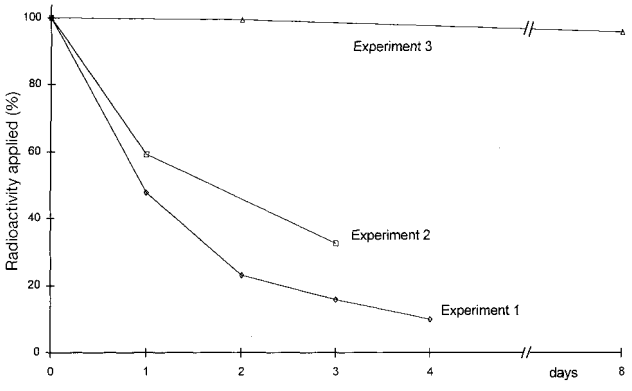

Figure 4. Photolysis of imidacloprid with incubation time. (◇) Experiment 1; DT_{50}; 0.7 days; radiation measured at leaves normalised to global radiation; 1020 J cm^{-2}; sum of global radiation measured at weather station; 6838 J cm^{-2}. (□) Experiment 2; DT_{50}; 1.4 days; radiation at leaves normalised to global radiation: 121 J cm^{-2}; sum of global radiation measured at weather station; 1460 J cm^{-2}. (△) Experiment 3 (in the dark).

proceeds via two routes. The first involves oxidation of the imidazoline ring, leading to the metabolite BNF 5540B, and the second involves a stepwise loss of the nitroimino group to give metabolite DIJ 9817.

The degradation rate depended very much on the amount of light falling on the leaf. There was very little degradation in Experiment 3 which was conducted in the dark (5% within eight days). By contrast, the time taken for 50% degradation of the applied compound (DT_{50}) under natural solar radiation was 0.7 and 1.4 days, respectively, in Experiments 1 and 2 (1.5th and second order, respectively). While the greater incident radiation in Experiment 1 led to a shorter DT_{50} value than for experiment 2, results for the latter experiment do not indicate a direct correlation between mean incident solar radiation (0.3–3.0 μm) and degradation. The degradation rates in the latter experiment were similar on day 1 and on days 2–3, even though the incident radiation on days 2–3 was 0.366 kJ cm^{-2} compared with 1.094 kJ cm^{-2} on day 1.

Conclusion

The photodegradation experiments reported here showed the presence of four compounds well-known as imidacloprid metabolites in plants. However, the identity of a new photometabolite, amounting to $\leqslant$3.8% of the applied radioactivity, was not established. Under field conditions, imidacloprid on tomato leaf surfaces is rapidly degraded, even under low light intensity conditions and it can be expected that degradation rates would be greater with greater exposure to light (greater angle of incidence of the sun, low latitudes, longer periods of daylight). The degradation of imidacloprid on leaf surfaces appears to be more complex than just photodegradation, although global radiation, presumably at certain specific wavelengths, plays a very important role.

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Selective mechanism of action of tebufenozide on lepidopteran cell lines

Meenakshi Sundaram,[1,2] Subba R Palli,[1]* PJ Krell,[2] Sardar S Sohi[1] and Arthur Retnakaran[1]

[1]*Great Lakes Forestry Centre, Canadian Forestry Service, Natural Resources Canada, PO Box 490, 1219 Queen Street East, Sault Ste Marie, Ontario P6A 5M7 Canada*

[2]*Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1 Canada*

Abstract: The non-steroidal ecdysone agonist, tebufenozide (RH-5992), induces a precocious incomplete molt primarily on lepidopteran insects, but has little or no effect on insects of other orders. 20 Hydroxyecdysone at 10^{-7} M induced the transcription factor CHR3 mRNA in CF-203 cells and DHR3 mRNA in DM-2 cells. Tebufenozide even at 10^{-10} M induced CHR3 mRNA in lepidopteran CF-203 cells, but even at 10^{-5} M it induced only trace levels of DHR3 mRNA in dipteran DM-2 cells. Studies using radiolabelled RH-5992 revealed that lepidopteran cell lines (CF-203 and MD-66) retained more of this compound within the cells than dipteran cell lines (DM-2 and K_c). The efflux of radiolabelled RH-5992 from DM-2 cells was temperature-dependent and was blocked by 10^{-5} M ouabain, an inhibitor of Na^+, K^+-ATPase, suggesting that the efflux was due to active transport.

Keywords: 20-hydroxyecdysone; tebufenozide; *Choristoneura* hormone receptor 3 (CHR3); *Drosophila* hormone receptor 3 (DHR3); ouabain

In the early 1980s, 1,2-diacyl-1-substituted hydrazines were found to induce precocious lethal molts in larvae. Among these, RH-5849 was the first compound that was shown to mimic the action of 20-hydroxyecdysone (20E) by acting through the ecdysteroid receptor.[1,2] Subsequently, tebufenozide (RH-5992), an analogue of RH-5849, was found to be very effective in inducing precocious lethal molts in lepidopteran larvae but had little or no effect on insects belonging to other orders. Two lepidopteran cell lines, FPMI-CF-203 (CF-203) and IPRI-MD-66 (MD-66) and two dipteran cell lines, DM-2 and K_c, were used to investigate the basis of lepidopteran specificity of tebufenozide *in vitro*. The mRNAs for hormone receptor 3 homologues (HR3) cloned from *Drosophila melanogaster* Meig (DHR3)[3] and *Choristoneura fumiferana* Clem (CHR3)[4] are expressed when ecdysteroid concentration rises during molts and are induced by treatment with 20E. Studies in several insects showed that HR3 mRNAs serve as

* Correspondence to: SR Palli, Great Lakes Forestry Centre, Canadian Forestry Service, Natural Resources Canada, PO Box 490, 1219 Queen Street East, Sault Ste Marie, Ontario P6A 5M7, Canada

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